Response of *Pithecellobium tortum* Martius (Leguminosae) Seeds to the Attack of the Phytophagous Braconid *Allorhogas dyspistus* Marsh (Hymenoptera: Braconidae)

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Abstract.—This work describes morphological changes on the seeds of *Pithecellobium tortum* caused by the braconid *Allorhogas dyspistus*, at Restinga (Costal scrub) of Barra de Maricá, Rio de Janeiro State, Brazil. This species was shown to be a gall maker on *P. Tortum* seeds whose galls result from the proliferation of parenchymatous cells near the tegument but not of the seed coat cells. Its impact on the host plant consists of decreases in plant reproductive potential not only by directly reducing seed viability, but also by contributing to seed mortality via the adult emergence hole which allows invasion by pathogenic micro-organisms. The braconid's way of eating the seed, keeping itself in a chamber apart from the seed embryo, which remains alive and therefore demanding nutrients, accords it the profile of a "manipulative parasite" in the sense of Weis & Abrahamson (1986).

Signs of insect herbivory on plants vary greatly. Some are simple feeding marks left on the host plant which normally do not involve any apparent morphological response. Other signs, however, are very complex, resulting from a noticeable morphological and/or physiological response of the plant. This response may be defensive, pathological or one which benefits the herbivore (Price 1980, Weis & Abrahamson 1986). Herbivores that are capable of manipulating the response of their host plant for their own benefit have been called "manipulative parasites" (Weis & Abrahamson 1986). Gall makers induce the development of localised growing structures resulting from the abnormal increase in number and/or size of plant cells (Darlington 1975). Normally, the galls are induced in undifferentiated tissues, which have their development manipulated

(Weis *et al.* 1988). The gall phenotype is the result of two genotypes: the one of the gall maker, responsible for the stimulus, and the other of the plant, which produces the reaction (Abrahamson & Weis 1987). From an evolutionary perspective, gall morphology is the product of natural selection on the insect stimulating the development of a structure for protection and nutrition and on the plant resisting or trying to avoid the insect stimulus (Weis *et al.* 1988).

Most of the known entomogenous galls are induced by Diptera (especially Cecidomyiidae), Hymenoptera, Homoptera and Thysanoptera (Meyer 1987; Shorthouse and Rohfritsch 1992). Within the Hymenoptera the Cynipidae is the most important family, but there are also records for Tenthredinidae, Eurytomidae, Eulophidae, Pteromalidae and Tanaostig-

matidae (Fernandes 1987). Guimarães (1957) reared *Allorhogas muesebecki* Guimarães from *Anemopaegma mirandum* Alph. DC. (Bignoniaceae) galls. Because this braconid was the only species to emerge the author concluded that this was the gall inducer. Since the first record of a phytophagous braconid (Macêdo & Monteiro 1989) and its specific description by Marsh (1991) as *Allorhogas dyspistus*, Infante *et al.* (1995) redescribed *Monitoriella elongata* Hedqvist and recorded it as a new case of phytophagy within the Braconidae. This species, like *A. dyspistus*, belongs to the Doryctinae, a group which, according to Wharton (1993), should be searched for more cases of phytophagy. Ramirez & Marsh (1996) described two new *Psenobolus* species (Braconidae: Doryctinae) which appear to develop as inquilines on plant tissue in fig flowers after their pollination by Agaonidae wasps. More recently, Austin & Dangerfield (1998) recorded the biology of *Mesostoa kerri* Austin and Wharton, a member of the endemic Australian subfamily Mesostoinae, as a new case of galling Braconidae.

Pithecellobium tortum Martius (Leguminosae) seeds are enclosed in fruits containing about 30 seeds side by side. They are attacked by the braconid *Allorhogas dyspistus* Marsh, which oviposits directly into immature seeds, when abundant endosperm and a small embryo are still present. After oviposition by the braconid the seed divides internally and externally, resulting in an intact region, joined to the funicle, where the seed embryo is usually found (Macêdo & Monteiro 1989). In many cases this region of the seed continues growing even after the adult insect has emerged. More than one *A. dyspistus* can be found in a single seed. In these cases, more than one division occurs and still a single intact region within the seed embryo is found. The main purpose of this study is to describe and discuss the morphological seed changes caused by *Allorhogas dyspistus* in *Pithecellobium tortum* and

to evaluate this impact of the insect on the host plant.

MATERIALS AND METHODS

All seeds were collected at Restinga (Coastal scrub) de Barra de Maricá (22°57'S and 12° 52'W), Maricá county, Rio de Janeiro state, Brazil.

To investigate seed tissue morphology, intact and attacked seeds were collected during June 1994, and fixed in 70% alcohol. Seeds were then laid in 10% ethylenediamine for one week, as suggested by Carlquist (1982) to soften them. After washing in distilled water three times for a 2 hours period each time, the seeds were dehydrated in ethanolic series and then embedded in paraffin wax following Johansen (1940). Longitudinal serial 15 µm sections were obtained with a rotary microtome and stained with basic blue astrafucsin (Roeser 1962).

In 1994, a further sample of 2990 seeds from 150 fruits obtained from five *P. tortum* individuals were collected and dissected in order to evaluate the rate of *A. dyspistus* attack and to check if the attacked seeds died or continued development until complete maturation. In 1995, 327 fruits from 12 plant individuals were also collected and dissected to evaluated *A. dyspistus* seed attack rate.

At the end of the 1995 fruiting season in June, mature fruits of *P. tortum* were collected from their parent plant and from the ground. Attacked and non-attacked seeds were then obtained to perform the tetrazolium viability test (Delouche *et al.* 1962). This test was performed immediately after the collection of seeds from the plant and from the ground, and also after three and eight months of laboratory storage at room temperature for seeds collected from the ground. All tested seeds were cut lengthwise and one half of each seed was completely immersed in 0.5% chloride of 2,3,5-triphenyl tetrazolium solution and the other half boiled before being submitted to this tetrazolium test of viability.
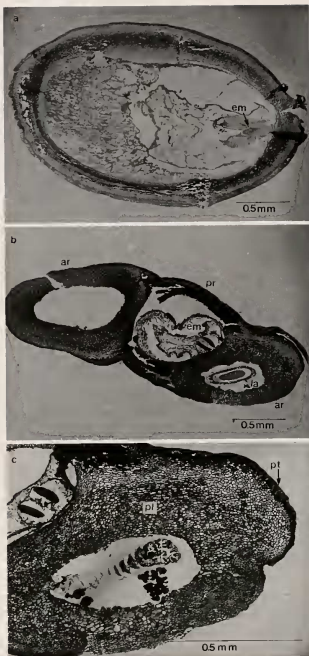
Fig. 1. Longitudinal sections of *Pithecellobium tortum* seeds. a) Non-attacked seed with its embryo (em); b) seed attacked by two *Allorhogas dyspistus* individuals, showing two attacked regions (ar); a larva (la) can be observed in one of them. The embryo (em) is present at the center in the preserved region (pr); c) an attacked region showing the seed coat palisade tissue (pt) which does not cover all of the region involving the larva. Note the thicker parenchymatous layer (pl).

This procedure was necessary in order to eliminate the possibility that the red coloration of the embryo was due to reduced ions and not to hydrogenases produced during the respiration process of the living embryo. If the dead boiled embryo coloured, the test would be invalid. All beakers were kept in darkness at room temperature and the result checked after 12 hours.

RESULTS

Comparison between non-attacked (Fig. 1a) and attacked (Fig. 1b) seeds shows that the tissue associated with *A. dyspistus* larvae probably results from the proliferation of the fundamental parenchymatous cells near the inside tegument. The gall, the region resulting from tissue proliferation, keeps the insect larva apart from the seed embryo through a clear division of the seed. The seed coat does not cover the entire proliferated region where the braconid larva is found (Fig. 1c). This can also be seen by the naked eye because the texture of this attacked region is clearly different from that where the embryo is found.

Allorhogas dyspistus attacked 55.85% of the dissected seeds. In all, only 6.1%, out of 1670 attacked seeds appeared healthy after insect emergence or death. All other attacked seeds died mainly because of contamination, probably fungus, which probably entered the seed through the *A. dyspistus* or its parasitoid exit holes.

All mature attacked and non-attacked seeds collected from the plant itself were viable according to the tetrazolium test, as well as all mature non-attacked seeds collected from the ground (Table 1). A smaller proportion of attacked seeds were viable in the three-months stored group and an even smaller proportion of the attacked seeds were viable in the eight-months stored group. It is worth noting that in all groups the embryos from the intact seeds exhibited a clearly darker pink coloration in comparison with the viable embryos from the attacked seeds. No boiled seeds were coloured thus validating the above results.

Allorhogas dyspistus seed attack reached more than 70% in five out of the 12 plants

Table 1. Percentage of viable seeds according to tetrazolium test of attacked and non-attacked seeds under four situations of collection and storage. The number of tested seeds is in parentheses.

Collection/situation of the tested seeds	Non-attacked seeds	Attacked seeds
From plant/immediately after collection	100 (50)	100 (22)
Ground/immediately after collection	100 (25)	100 (18)
Ground/after three months of storage	100 (20)	73 (15)
Ground/after eight months of storage	100 (6)	18 (11)

Table 2. Numbers of seeds collected (N), attacked seeds and percentage of seed attack by *Allorhogas dyspistus* on each of the 12 plant individuals sampled in 1995.

Plant	N seeds	Attacked seeds	% attack *A. dyspistus*
1	359	41	11.42
2	288	33	11.46
3	386	130	33.68
4	339	83	24.48
5	252	26	10.32
6	365	290	79.45
7	394	274	69.54
8	558	39	6.99
9	385	307	79.74
10	468	436	93.16
11	436	435	99.77
12	338	337	99.70

studied and in two of these the rates were very near 100% (Table 2).

DISCUSSION

Clear cell proliferation, characteristic of the process of gall formation, indicates that *A. dyspistus* induces galls in *P. tortum* immature seeds. This kind of gall is, according to Gagné (1994), a simple gall, which does not differ from the normal plant tissue except for cell proliferation. This is very different from most of the complex galls (*sensu* Gagné 1994) on plant vegetative parts (*e.g.* Redfern & Askew 1992). *Monitoriella elongata* (Infante *et al.* 1995) and *Mesostoa kerri* (Austin and Dangerfield 1998), are two Braconidae leaf gallers which show galls are apparently as complex as any Cecidomyiidae leaf gall and quite different from the simple galls of *A. dyspistus*. As seeds are attacked when immature and still in the process of development, meristematic tissue is available, and this is where galls are primarily produced (Mani 1964, Bronner 1977). Quicke (1997) points out that there is growing evidence that at least seed predation actually involves the stimulation of proliferation of some plant cells, that is, a kind of incipient gall formation or simple gall (*sensu* Gagné 1994).

Other insect groups which develop inside seeds, such as Bruchidae (Coleoptera), do not promote any similar response

and most of them feed upon embryo and cotyledons (Southgate 1979). Even *Rinochenus stigma* (L.) (Coleoptera; Curculionidae), which eats the cotyledons but not the embryo of *Hymenaea* (Leguminosae) seeds, does not induce any kind of seed division (T.M. Lewinsohn pers. com.).

The way of attack by the braconid leaving the seed embryo region intact may be considered a way of maintaining nutrient demand by the living seed and therefore for the insect, which could not survive otherwise. The aggregated pattern of *A. dyspistus* occurrence in the fruits (M.V. Macêdo, pers. obs.) could, if the seeds died, cause selective abortion of most attacked fruits, as in *Cassia grandis* L. (Leguminosae) attacked by bruchids (Janzen 1971). This negative effect for the insect is more evident in younger fruits (Stephenson 1981). Thus, *A. dyspistus* may be considered a manipulative parasite of *P. tortum* immature seeds (*sensu* Weis & Abrahamson 1986).

It is clear that the potential negative effect is very high where reproductive tissue is attacked (Abrahamson & Weis 1987). Reduction of *P. tortum* seed viability over time is a clear effect of *A. dyspistus* attack (Table 1). Furthermore, the capability of germination of attacked living seeds still

remains to be tested. The tetrazolium test only proved that attacked seeds are alive; these seeds, however, could have lower chances of germination and establishment, as may be suggested by attacked seeds having a lower degree of respiratory activity which was shown by their lighter coloration in the test. Moreover, *A. dyspistus* exit holes seem to serve as a communicating channel between the seed and the external environment, which makes it possible for micro-organisms to enter and eventually kill most of the attacked seeds.

This work shows that, directly or indirectly, *A. dyspistus* considerably reduces plant fitness, and, because the species may attack up to 100% of the seeds in a plant, it can be considered a good example of a galler that imposes high negative effects on its host plant. We suggest that the galler's behaviour of maintaining the seed embryo alive while feeding upon seed tissue may avoid abortion of immature seeds increasing the galler's chances of survival.

ACKNOWLEDGMENTS

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